Effects of Low Phytate Barley (*Hordeum vulgare* L.) on Zinc Utilization in Young Broiler Chicks

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ABSTRACT Two 21-d experiments were conducted to evaluate the effects of low phytate barley (LPB) on Zn utilization by young broiler chicks and to determine the contribution of endogenous phytase, present in LPB. In the first experiment, ninety-six 1-d-old male chicks were assigned to a 2 × 3 factorial arrangement of treatments (4 pens of 4 chicks/treatment). Factors were barley type [wild-type barley (WTB) and LPB mutant M 955] and supplemental Zn (0, 10, or 20 mg of Zn/kg). In the second experiment, two hundred forty 1-d-old straight-run broiler chicks were assigned to a 2 × 2 × 3 factorial arrangement of treatments (4 pens of 5 chicks/treatment). Factors were barley type (WTB and LPB), autoclave treatment [nonautoclaved or autoclaved (121°C, 20 kg/cm^2, 20 min)], and supplemental Zn (0, 10 or 20 mg of Zn/kg). Barley made up 60% of the diets and was the only source of phytate. On average, basal diets contained 26 mg of Zn/kg. Feed intake and body weight gain were greater ($P < 0.05$) in broilers fed LPB compared with WTB in experiment 2. Zinc concentration in toes and tibias were affected ($P < 0.0001$) by barley type (LPB > WTB) and supplemented Zn levels (20 > 10 > 0 mg of Zn/kg), and significant barley type × Zn interactions were also observed in both experiments. Substitution of LPB for WTB increased tibia and toe Zn by 46 and 25%, respectively, an increase comparable to that achieved with supplementing the diet with 20 mg of Zn/kg. No effect of autoclaving was observed for any variable in experiment 2. Retention of P and Zn was higher ($P < 0.001$) in chicks fed LPB compared with WTB in both experiments. Zinc retention was influenced ($P < 0.0001$) by dietary Zn, and barley type × Zn level interactions ($P < 0.05$) were observed in both experiments. Chicks fed LPB utilized more dietary Zn and P than those fed WTB, and this improved mineral utilization was not due to endogenous phytase present in barley.

Key words: phytate, barley, zinc, chick, autoclaving

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INTRODUCTION

Phytic acid consists of a phosphorylated myo-inositol ring and has high chelation capacity for multivalent cations such as zinc, calcium, copper, iron, magnesium, and aluminum (Cheryan, 1980). Zinc is probably the mineral that is most susceptible to phytate complexation (Kornegay, 2001). In research with humans, chicks, swine, and rats it was observed that dietary Zn requirement was increased by dietary phytate (O'Dell and Savage, 1960; Lease, 1966; Atwal et al., 1980; Morris and Ellis, 1980; Lo et al., 1981; Lonnerdal et al., 1989). Morris (1986) and Oberleas and Harland (1996) reported results that clearly showed that phytate was a significant factor in the development of Zn deficiency in rats and chicks. The presence of Ca aggravated the negative effect of phytate on Zn utilization, probably through the formation of an insoluble Ca-phytate-Zn complex, which prevents the absorption of Zn in rats (Oberleas et al., 1966).

The addition of microbial phytase, an enzyme that hydrolyzes phytate, increases the bioavailability of phytate P, cations and protein in monogastric diets (Ravindran et al., 1995; Kies et al., 2001). Levels of supplemental phytase between 600 and 800 **PPU** (1 PPU = the amount of enzyme required to liberate 1 n*M* inorganic P from 2 m*M* sodium phytate per minute at pH 5.0 and 40°C)/kg of diet have shown the best results for improving P digestibility and retention in pigs and poultry (Kornegay, 2001). Diets formulated using ingredients having high endogenous phytase activity, such as wheat, barley, and triticale also promote greater absorption of phosphorus (Eeckhout and De Paepe, 1994). The addition of 800 PPU/kg of microbial phytase to broiler diets increased Zn retention and decreased Zn excretion (Thiel and Weigand, 1992). Biehl et al. (1995) reported that phytase and dihydroxycholecalciferol supplementation increased growth rate and tibia Zn concentration in chicks.

Another approach for improving P and Zn utilization is to develop feedstuffs with lower levels of phytic acid.

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Recently, USDA scientists developed several low phytate barley (**LPB**) mutants with reduced phytate P (50 to 90%) with no change in total P (Raboy, 2001). In recent studies, Guaiume et al. (2002) reported higher P and Zn retention in chicks fed LPB compared with wild-type barley (**WTB**), and Li et al. (2001a) reported that P availability from LPB was estimated to be 49% compared with 38% for WTB. Egli et al. (2004) reported that adult humans absorbed more Zn from a dephytinized wheat-soy diet compared with the nondephytinized diet (34.6 vs. 22.8%, respectively). Similarly, Adams et al. (2002) reported that substitution of normal corn by low phytate (**LP**) corn in a corn-based diet resulted in a substantial increase in Zn absorption by healthy adult humans.

The objectives of these studies were to evaluate the effects of LPB, which contains 90 to 95% less phytate P, on Zn and P utilization by young broiler chicks and to determine the contribution of endogenous phytase present in LPB on Zn and P utilization.

MATERIALS AND METHODS

In both experiments, chicks were purchased from a commercial hatchery (Stover Hatchery, Stover, MO) and raised in stainless steel chick batteries in a temperature-controlled room. Chicks were maintained on a 24 h constant-light schedule and allowed ad libitum access to feed and tap water. Zinc concentration of tap water ranged from 0.03 to 0.41 mg/L with an average of 0.21 mg/L. Mortality was recorded as it occurred. The basal diet was a barley (*Hordeum vulgare* L.)-casein-glucose diet formulated to meet or exceed the nutritional requirements (except for Zn) of growing chicks as recommended by NRC (1994). The analytical composition of the barleys is presented in Table 1, and the composition of the basal diets is presented in Table 2. Ground samples were analyzed for dry matter, ash, crude protein, and crude fiber (AOAC, 2000). Calcium, magnesium, iron, manganese, copper, and zinc were analyzed by flame atomic spectrophotometry. Amino acid content was measured by sample hydrolysis with quantification by HPLC (AOAC, 2000). The LPB used in this study was the M 955 mutant isolated from the cultivar Harrington used here as a check on the validity of the mutation. The M 955 LPB produces grain that has similar levels of total P when compared with the WTB control but contains 90 to 95% less phytate P (Dorsch et al., 2003; Ockenden et al., 2004). The LPB and WTB used in these experiments were provided by the USDA-Agricultural Research Service, Small Grains and Potato Research Unit, Aberdeen, ID. Chromic oxide was included in the diets as an inert marker at a concentration of 0.05% for determination of mineral retention. Ronozyme B [(endo-1,3(4)-β-glucanase, 140 **FBG**/g (1 FBG unit = the amount of enzyme that releases 1.0 μM of glucose or reducing carbohydrates per minute at pH 5.0 and 30°C); endo-1,4-β-xylanase, 600 **FXU**/g (1 FXU = the amount of enzyme that releases 7.8 μM of reducing xylose equivalents from azo-wheat arabinoxylan per minute at pH 6.0 and 50°C); α-amylase, 50 **KNU**/g (1 KNU = the amount of enzyme that breaks down 5.26 g of starch per h at pH 7.1 and 37°C)] was also included at a level of 500 g/ton to avoid confounding effects caused by nonstarch polysaccharides. Endogenous phytase activity in the diets was measured according to Zyla et al. (2002). Barley was the only source of phytate in the diets. Phytate content of the barleys was determined according to Dorsch et al. (2003).

Table 1. Analytical nutrient content of wild-type barley (WTB) and low phytate barley (LPB) used in experiments 1 and 2

	Barley type	
Nutrient	WTB	LPB
TME,[1] kcal/kg	3,137	3,170
CP, %	12.97	13.34
Crude fat, %	1.73	1.15
Crude fiber, %	3.65	3.78
Ash, %	2.30	2.26
Moisture, %	11.09	11.31
Amino acid content, %		
Arginine	0.53	0.67
Cysteine	0.25	0.28
Glycine	0.40	0.49
Histidine	0.23	0.29
Isoleucine	0.37	0.45
Leucine	0.73	0.90
Lysine	0.37	0.47
Methionine	0.18	0.22
Phenylalanine	0.55	0.68
Proline	1.06	1.33
Serine	0.36	0.44
Threonine	0.33	0.40
Tryptophan	0.15	0.15
Tyrosine	0.26	0.32
Valine	0.51	0.63
Macro and micro mineral content		
Ca, %	0.047	0.049
Mg, %	0.12	0.12
Fe, mg/kg	61.70	71.00
Mn, mg/kg	16.40	15.40
Cu, mg/kg	3.40	3.60
Zn, mg/kg	23.00	24.40
Analyzed P content, %		
Phytate P	0.238	0.005
Nonphytate P	0.125	0.347
Total P	0.363	0.352

[1]Data are means of 6 replicate pens of 1 rooster each.

Experiment 1

A total of ninety-six 1-d-old male chicks was randomly assigned to a 2 × 3 factorial [2 barley types (WTB or LPB) and 3 Zn levels (0, 10, or 20 mg of Zn/kg)] arrangement of treatments with 4 pen replicates of 4 chicks assigned to each of the 6 dietary treatments for 3 wk. Zinc was supplied as $ZnSO_4$.

Experiment 2

A total of two hundred forty 1-d-old straight-run broiler chicks were randomly assigned to a 2 × 2 × 3 factorial [2 barley types (WTB or LPB), autoclaved (121°C, 20 kg/cm^2, 20 min.) or nonautoclaved, and 3 Zn levels (0, 10, or 20 mg of Zn/kg)] arrangement of treatments with 4 pen replicates of 5 chicks assigned to each of the 12 dietary treatments for 3 wk. Zinc was supplied as $ZnSO_4$.

Table 2. Composition of wild-type barley (WTB) and low phytate barley (LPB) diets fed to broilers in experiments 1 and 2

	Experiment 1		Experiment 2	
Ingredient	WTB basal (%)	LPB basal (%)	WTB basal (%)	LPB basal (%)
WTB	60.000	—	60.000	—
LPB	—	60.000	—	60.000
Casein	15.199	14.769	15.323	15.063
Glucose	12.181	12.181	11.846	11.846
Corn oil	5.589	5.589	5.731	5.731
Fish meal[1]	3.500	3.500	3.500	3.500
Limestone	1.704	1.704	1.725	1.725
KH_2PO_4	0.483	—	0.483	—
Salt	0.448	0.448	0.387	0.387
Sand	—	0.913	0.233	0.976
L-Arginine	0.189	0.189	0.178	0.178
K_2CO_3	0.102	0.102	0.102	0.102
Trace mineral mix[2]	0.100	0.100	0.100	0.100
$CaHPO_4$	0.114	0.114	0.098	0.098
Choline	0.191	0.191	0.094	0.094
Vitamin mix[3]	0.050	0.050	0.050	0.050
Selenium premix[4]	0.050	0.050	0.050	0.050
Ronozyme B	0.050	0.050	0.050	0.050
Chromic oxide	0.050	0.050	0.050	0.050
Nutrients calculated				
ME (kcal/kg)	3,200.00	3,200.00	3,200.00	3,200.00
CP (%)	23.00	23.00	23.00	23.00
Ca (%)	1.00	1.00	1.00	1.00
Nonphytate P (%)	0.45	0.45	0.45	0.45
Nutrients analyzed				
Ca (%)	0.92	0.94	0.99	1.04
Total P (%)	0.55	0.44	0.55	0.45
Zn (mg/kg)	24.08	24.96	27.42	28.13
Endogenous phytase activity (PPU[5]/kg)	110.00	114.00	110.00	114.00

[1]Fish meal 60.5% CP.

[2]Trace mineral mix provided (mg/kg of diet): manganese, 140 mg from MnO_2; iron, 130 mg from $FeSO_4 \cdot 7H_2O$; copper, 12 mg from $CuSO_4$; iodine, 0.7 mg from ethylenediamine dihydroiodide.

[3]Vitamin mix supplied (per kg of feed): vitamin A (retinyl acetate), 8,810 IU; cholecalciferol, 3,855 IU; vitamin E (DL-α-tocopheryl acetate), 14 IU; niacin, 55 mg; calcium pantothenate, 17 mg; riboflavin, 6.6 mg; pyridoxine, 2.2 mg; menadione sodium bisulfite, 1.7 mg; folic acid, 1.4 mg; thiamin mononitrate, 1.1 mg; biotin, 0.2 mg; cyanocobalamine, 11 μg; and ethoxyquin, 83 mg.

[4]Selenium premix provided 0.20 mg of Se/kg of diet from $NaSeO_3$.

[5]1 PPU is the amount of enzyme required to liberate 1 n*M* inorganic P from 2 m*M* sodium phytate per minute at pH 5.0 and 40°C.

Measurements

During wk 3, excreta samples (4 pens/treatment) were collected for 5 consecutive days to determine P, Ca, and Zn retention. Daily excreta output was homogenized, and a 10% aliquot was collected, pooled, and frozen for subsequent analysis. At the end of the experiments, chicks were weighed by pen. Feed consumption was recorded at the same time, and feed conversion was calculated.

Chicks were then euthanized with carbon dioxide, and the middle toe from each foot and right tibia (3 chicks per pen) were collected.

For toe ash determination, toes were dried at 100°C for 24 h, weighed, and dry ashed in a muffle furnace at 540°C overnight. For tibia ash determination, right tibia were stripped of adhering tissue following immersion in boiling water, ether extracted, dried at 100°C for 24 h, weighed, and dry ashed in a muffle furnace at 540°C overnight. These procedures for bone ash determination are similar to those reported previously (Potter et al., 1995; Garcia and Dale, 2006) except that samples were ashed at 540°C to prevent zinc volatilization. Excreta and feed samples were dried at 60°C and ground through a 1-mm stainless steel screen. Bone ash and duplicate samples of excreta and feed were digested by nitric-perchloric acid wet digestion. Phosphorus concentrations in feed and excreta were determined colorimetrically by the molybdo-vanadate method (AOAC, 2000). Calcium, Zn, and Cr concentrations in the bone, feed and excreta were determined by flame atomic absorption spectrophotometry, and the assay was validated by including standard reference material (peach leaves) from the National Institute of Standards and Technology. Phosphorus, Ca, and Zn retention were calculated using the following formula: 100% − [100% × (Cr concentration in feed or excreta) × (P or Ca or Zn concentration in feed or excreta)].

The animal care and use protocol for these studies was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

Table 3. Effects of wild-type barley (WTB) and low phytate barley (LPB) and levels of added dietary Zn on performance of broilers to 21 d of age (experiment 1)[1]

Barley type	Zn (mg/kg)	Feed intake (g)	BW gain (g)	Feed to gain (g:g)
WTB	0	765	530	1.44
WTB	10	863	605	1.43
WTB	20	842	592	1.42
LPB	0	833	593	1.41
LPB	10	848	585	1.45
LPB	20	870	602	1.45
Pooled SEM		23	17	0.03
Source of variation			*P*-value	
Barley (B)		0.1722	0.2231	0.8268
Zn		0.0374	0.0821	0.9184
B × Zn		0.2338	0.0675	0.6031
Main effect mean				
B				
WTB		823	576	1.43
LPB		851	593	1.44
Zn	0	799[b]	561	1.43
	10	856[a]	595	1.44
	20	856[a]	597	1.43

[a,b]Values within a column with no common superscript are significantly different ($P < 0.05$).

[1]Data are means of 4 replicate pens of 5 chicks per pen.

Statistical Analysis

Data were analyzed as a 2 × 3 factorial (experiment 1) and 2 × 2 × 3 factorial (experiment 2) by ANOVA using the GLM procedures of SAS (SAS Institute, 1996). The means for treatments showing significant differences in the ANOVA were compared using Fisher's protected least significant difference procedure. Statistical significance was accepted at $P \leq 0.05$.

RESULTS

Experiment 1

No barley type × Zn level interactions were observed ($P > 0.05$) for performance data (Table 3). Feed intake, body weight gain, and feed conversion were not affected ($P > 0.05$) by barley type. However, chicks fed diets with 10 and 20 mg/kg of supplemental Zn had higher ($P < 0.05$) feed intake and body weight gain than chicks fed diets with no supplemental Zn.

No barley type × Zn level interaction ($P > 0.05$) was observed for tibia or toe ash (Table 4). Percent toe ash was affected ($P < 0.05$) by barley type. However, percent tibia ash was not affected ($P > 0.05$) by barley type (Table 4). Supplemental Zn did not affect ($P > 0.05$) percent toe or tibia ash. However, Zn concentration in toes and tibias increased ($P < 0.0001$) with increasing levels of dietary Zn and was higher ($P < 0.0001$) in chicks fed LPB compared with those fed WTB. Significant barley type × Zn level interactions ($P < 0.0001$) were observed for toe Zn (**TZ**) and tibia Zn (**TBZ**; Table 4). In chicks fed WTB, toe Zn and tibia Zn increased with increasing Zn supplementation, whereas the consistently high levels of toe Zn and tibia Zn in chicks fed LPB were not affected by higher concentrations of supplemental Zn.

Phosphorus retention was higher ($P < 0.0001$) in chicks fed LPB compared with chicks fed WTB but was not affected by dietary Zn (Table 5). Zinc retention was higher ($P < 0.001$) in chicks fed LPB but declined with increasing Zn supplementation. A significant barley type × Zn level interaction ($P < 0.0001$) was also observed for Zn retention (Table 5).

Experiment 2

No interactions between barley type, Zn level, or autoclaving were observed for performance variables (Table 6). Feed intake and body weight gain were higher ($P < 0.05$) in chicks fed LPB compared with chicks fed WTB but were not affected ($P > 0.05$) by Zn level or autoclaving process (Table 6). Feed conversion was not affected ($P > 0.05$) by dietary treatments.

Percent toe ash and percent tibia ash of chicks were not affected ($P > 0.05$) by dietary treatments (Table 7). However, zinc concentration in toes and tibias increased ($P < 0.0001$) with increasing levels of dietary Zn and were higher ($P < 0.0001$) in chicks fed LPB compared with those fed WTB (Table 7). In chicks fed WTB, toe Zn and tibia Zn increased with increasing Zn supplementation, whereas similar to results in experiment 1, in chicks fed LPB increasing Zn supplementation did not further increase the already high toe and tibia Zn. This resulted in a significant ($P < 0.0001$) barley type × zinc level interaction (Table 7). No main or interactive effects of autoclaving were observed for bone ash.

Chicks fed LPB retained more P ($P < 0.0001$) than chicks fed WTB (Table 8). Calcium retention in broiler chicks was not affected ($P > 0.05$) by barley type. However, Ca retention was lower ($P < 0.05$) in chicks fed 20 mg of Zn/kg compared with chicks fed 0 or 10 mg of Zn/kg (Table 8). Chicks fed LPB retained more Zn ($P < 0.001$) than chicks fed WTB. Zinc retention decreased ($P < 0.0001$) with zinc supplementation (Table 8). The difference in Zn retention between the 2 barley types was much greater at zero compared with 10 or 20 mg of supplemental Zn/kg, resulting in a significant ($P < 0.05$) barley type × Zn level interaction (Table 8). No main or interactive effects of autoclaving were observed for P, Ca, or Zn retention.

DISCUSSION

Performance was not affected by barley type in experiment 1 but was affected by barley type in experiment 2, with chicks fed LPB having higher feed intake and body weight gain (942 and 694 g, respectively) compared with chicks fed WTB (877 and 648 g, respectively). Results of experiment 1 are consistent with previous reports in turkeys (Li et al., 2001b), pigs (Veum et al., 2001), and fish (Sugiura et al., 1999), whereas results of experiment 2 are inconsistent with these previous reports. Because diets in both experiments theoretically contained the same levels of nonphytate P, differences in growth performance in experiment 2 were unexpected. Jang et al. (2003) also reported no differences in growth in chicks fed LPB and

Table 4. Effects of wild-type barley (WTB) and low phytate barley (LPB) and levels of added dietary Zn on broiler tibia and toe ash and tibia and toe Zn content at 21 d of age (experiment 1)[1]

Barley type	Zn (mg/kg)	Tibia ash (%)	Toe ash (%)	Tibia Zn (mg/kg)	Toe Zn (mg/kg)
WTB	0	46.45	13.41	98.80	45.69
WTB	10	46.19	13.64	163.61	63.41
WTB	20	46.03	13.31	189.28	67.75
LPB	0	45.96	12.68	214.66	70.15
LPB	10	46.23	13.21	206.40	73.86
LPB	20	45.71	12.81	213.81	73.17
Pooled SEM		0.41	0.28	5.46	1.31
Source of variation		*P*-value			
Barley (B)		0.4588	0.0263	<0.0001	<0.0001
Zn		0.6402	0.3283	<0.0001	<0.0001
B × Zn		0.8074	0.8596	<0.0001	<0.0001
Main effect mean					
B					
WTB		46.22	13.45[a]	150.56[b]	58.95[b]
LPB		46.97	12.90[b]	211.63[a]	72.39[a]
Zn	0	46.20	13.04	156.73[c]	57.92[b]
	10	45.87	13.42	185.00[b]	68.63[a]
	20	46.21	13.05	201.54[a]	70.46[a]

[a–c]Values within a column with no common superscript are significantly different ($P < 0.05$).

[1]Data are means of 4 replicate pens of 5 chicks per pen.

WTB when available P was used as a covariate in the statistical model. A likely explanation for the difference between the 2 experiments is that the higher feed intake and body weight gain in experiment 2 increased the demand for available nutrients. This demand was met to a greater extent by the LPB diet compared with the WTB diet.

The NRC (1994) states that 40 mg of zinc/kg of diet is optimal for chick growth; therefore in these experiments, dietary levels of zinc were used that ranged from deficient (24 mg/kg) to adequate (48 mg/kg). Results of experiment 1 indicated that the basal diet (~24 mg of Zn/kg) required an additional 10 mg of Zn/kg to maximize chick performance. In contrast, in experiment 2, Zn level in the basal diet (~27 mg/kg) was enough to meet the requirement for chick growth because additional Zn did not further improve chick growth. Similar results to that of experiment 2 were reported by Guaiume et al. (2002), who observed no difference in growth performance of chicks fed diets containing WTB and LPB and supplemented with 0, 10, and 20 mg of Zn/kg of diet. These results suggest that the Zn requirement of chicks fed a barley-based diet may be less than 40 mg/kg but higher than 24 mg/kg. However, Mohanna and Nys (1999) reported that a dietary Zn concentration of 45 mg/kg gave the best performance in broilers fed corn-soybean meal-wheat diets from hatch to 21 d of age.

Table 5. Effects of wild-type barley (WTB) and low phytate barley (LPB) and levels of added dietary Zn on retention of P and Zn in broilers to 21 d of age (experiment 1)[1]

Barley type	Zn (mg/kg)	P retention (%)	Zn retention (%)
WTB	0	67.42	42.48
WTB	10	64.75	37.23
WTB	20	61.55	28.22
LPB	0	74.06	62.98
LPB	10	72.62	43.28
LPB	20	73.42	33.52
Pooled SEM		1.75	1.34
Source of variation		*P*-value	
Barley (B)		<0.0001	<0.0001
Zn		0.2010	<0.0001
B × Zn		0.3192	<0.0001
Main effect mean			
Barley			
WTB		64.57[b]	35.98[b]
LPB		73.37[a]	46.60[a]
Zn	0	70.74	52.45[a]
	10	68.68	37.53[b]
	20	67.49	21.51[c]

[a–c]Values within a column with no common superscript are significantly different ($P < 0.05$).

[1]Data are means of 4 replicate pens of 5 chicks per pen.

Percentage tibia ash was not affected by dietary treatments in either experiment. In experiment 1, percent toe ash in the WTB treatment was higher than in the LPB treatment. However, there was no significant difference in toe ash weight (mg) between barley types (462 and 453 mg of toe ash for WTB and LPB, respectively). These contrasting results may be due to the influence of soft tissue (cartilage and skin) present on the toes, on percent toe ash determination. In contrast, toe ash weight would not be influenced by soft tissue. However, in previous studies with turkey poults (Li et al., 2001b) and broiler chicks (Linares et al., 2003) fed diets containing NRC levels of nonphytate P, no differences in percent tibia or toe ash were observed between chicks fed WTB or LPB. Research with chicks fed LP corn have also shown bone mineralization equal to or superior to those fed diets with normal corn (Li et al., 2000; Waldroup et al., 2000; Yan et al., 2000; Jang et al., 2003) and equal levels of nonphytate P.

As expected, no effects of Zn level on percentage of bone ash in chicks were observed. Because Ca and P are

Table 6. Effects of autoclaving of wild-type barley (WTB) and low phytate barley (LPB) and levels of added dietary Zn on performance of broilers to 21 d of age (experiment 2)[1]

Barley type	Autoclaved	Zn (mg/kg)	Feed intake (g)	BW gain (g)	Feed to gain (g:g)
WTB	No	0	843	616	1.37
WTB	No	10	851	606	1.40
WTB	No	20	902	665	1.36
WTB	Yes	0	846	635	1.33
WTB	Yes	10	926	690	1.34
WTB	Yes	20	896	674	1.33
LPB	No	0	956	702	1.36
LPB	No	10	960	699	1.37
LPB	No	20	925	690	1.34
LPB	Yes	0	940	689	1.37
LPB	Yes	10	945	693	1.37
LPB	Yes	20	928	691	1.35
Pooled SEM			27	21	0.03
Source of variation				*P*-value	
Barley (B)			0.0002	0.0005	0.9111
Autoclaved (A)			0.6488	0.2054	0.2225
Zn			0.4358	0.4272	0.4161
B × A			0.2887	0.0816	0.2014
B × Zn			0.1523	0.2697	0.9254
A × Zn			0.5930	0.3956	0.7842
B × A × Zn			0.4267	0.3838	0.9477
Main effect means					
B					
WTB			877[b]	648[b]	1.36
LPB			942[a]	694[a]	1.36
A					
No			906	663	1.37
Yes			913	679	1.35
Zn		0	896	660	1.36
		10	921	672	1.37
		20	913	680	1.34

[a,b]Values within a column with no common superscript are significantly different ($P < 0.05$).

[1]Data are means of 4 replicate pens of 5 chicks per pen.

the major components of bones and the levels of these minerals in the diets were kept at NRC (1994) recommended levels, minimal differences in dietary Zn content did not affect bone mineralization. In agreement with this study, Yi et al. (1996) and Mohanna and Nys (1999) also did not observe effects of Zn level on percentage of bone ash in broilers.

Toe and tibia Zn were higher in chicks fed LPB compared with those fed WTB in both experiments. These results are consistent with previous reports by Guaiume et al. (2002) and Jang et al. (2003) who also observed increased bone Zn in chicks fed LPB compared with those fed WTB. Increased levels of phytic acid in feed have been shown to cause a decrease in Zn absorption in humans (Adams et al., 2002; Egli et al., 2004) and a decrease in Zn concentration in rat femurs (Atwal et al., 1980; Morris and Ellis, 1980). The addition of 10 and 20 mg of Zn/kg to the low Zn diets resulted in an increase of TBZ content by 18 and 28% and TZ content by 18 and 22%, respectively, in experiment 1. In experiment 2, the addition of 10 and 20 mg of Zn/kg to the low Zn diets resulted in an increase of TBZ content by 20 and 38% and TZ content by 16 and 32%, respectively, but this increase was only observed in chicks fed WTB. Increased TZ and TBZ concentration with increasing dietary Zn observed in this study is consistent with previous studies (Biehl et al., 1995; Yi et al., 1996; Mohanna and Nys, 1999; Guaiume et al., 2002). The magnitude of change in TZ and TBZ content in response to increasing concentrations of dietary Zn confirms previous reports indicating that bone Zn measurements are sensitive indicators for determining Zn utilization.

Significant barley type × Zn level interactions were also observed for TZ and TBZ. In chicks fed WTB, toe Zn and tibia Zn increased with increasing Zn supplementation, whereas in chicks fed LPB, toe Zn and tibia Zn did not increase with supplemental Zn. These results indicate that the diet containing LPB with no supplemental Zn supplied enough Zn to maximize TZ and TBZ concentrations, whereas in the diets containing WTB, toe Zn and tibia Zn concentrations continued to increase with increasing levels of supplemental Zn. These results also confirm previous reports (Li et al., 2001a,b) on the increased availability of Zn in LPB compared with WTB.

Chicks fed LPB diets retained 13.6 to 16.8% more P than chicks fed WTB diets, indicating that the availability of P from LPB is greater than that from WTB. Similar results were observed in chicks (Li et al., 2001a; Guaiume et al., 2002; Jang et al., 2003), turkeys (Li et al., 2001b), pigs (Veum et al., 2001), and fish (Sugiura et al., 1999) fed LPB and WTB diets. The LPB used in this study (M 955) is the only grain or legume genotype currently avail-

Table 7. Effects of autoclaving of wild-type barley (WTB) and low phytate barley (LPB) and levels of added dietary Zn on broiler tibia and toe ash and tibia and Zn content at 21 d of age (experiment 2)[1]

Barley type	Autoclaved	Zn (mg/kg)	Tibia ash (%)	Toe ash (%)	Tibia Zn (mg/kg)	Toe Zn (mg/kg)
WTB	No	0	46.73	13.19	98.08	37.53
WTB	No	10	46.55	13.00	152.21	48.62
WTB	No	20	46.60	12.88	200.89	59.57
WTB	Yes	0	46.83	13.33	81.60	33.45
WTB	Yes	10	46.71	13.20	157.37	52.20
WTB	Yes	20	46.82	13.39	208.53	62.56
LPB	No	0	46.09	13.04	227.23	62.65
LPB	No	10	46.38	13.48	217.09	59.96
LPB	No	20	46.91	12.35	232.61	64.21
LPB	Yes	0	47.09	12.22	223.93	59.17
LPB	Yes	10	46.55	13.86	233.00	62.93
LPB	Yes	20	46.83	13.17	228.32	68.26
Pooled SEM			0.42	0.52	6.08	4.35
Source of variation			*P*-value			
Barley (B)			0.6585	0.3035	<0.0001	<0.0001
Autoclaved (A)			0.3800	0.8993	0.8268	0.6923
Zn			0.8283	0.8447	<0.0001	<0.0001
B × A			0.8064	0.4182	0.5722	0.9452
B × Zn			0.9180	0.6288	<0.0001	0.0069
A × Zn			0.5944	0.3418	0.0713	0.4124
B × A × Zn			0.4839	0.6446	0.2854	0.9903
Main effect mean						
B						
WTB			46.71	13.16	149.78[b]	48.99[b]
LPB			46.60	12.85	227.03[a]	62.86[a]
A						
No			46.54	12.99	188.02	55.43
Yes			46.76	13.03	188.79	56.43
Zn	0		46.68	12.95	157.71[c]	48.20[c]
	10		46.55	13.13	189.92[b]	55.93[b]
	20		46.72	12.95	217.59[a]	63.65[a]

[a–c]Values within a column with no common superscript are significantly different ($P < 0.05$).

[1]Data are means of 4 replicate pens of 5 chicks per pen.

able for research in which grain phytate is reduced by greater than 90%. Because barley was the only source of phytate in the experimental diets, differences in nutritional status or performance between chicks fed the WTB and LPB diets can, in a genetic sense, be attributed to the allelic difference in a single gene between WTB and LPB that results in this large reduction in grain phytate, and accompanying large increase in available P.

Phosphorus excretion decreased an average of 25.8% when LPB was used in chick diets, compared with WTB diets. This level of excreta P reduction is consistent with previous reports (Li et al., 2000, 2001b; Yan et al., 2000; Jang et al., 2003; Linares et al., 2003) in which reductions in P excretion ranged from 17 to 33% when poultry were fed diets containing LPB or LP corn compared with their wild-type counterparts. These reductions in excreta P concentrations are similar to reductions observed (28-30%) when phytase is used to replace 0.1 to 0.15% nonphytate P in broiler diets (Waldroup et al., 2000; Yan et al., 2000).

Zinc retention was significantly higher in chicks fed LPB (averaging 27.5% for both experiments) compared with chick fed WTB. Guaiume et al. (2002) observed similar results in chicks fed LPB and WTB and the same levels of supplemental Zn. Thiel and Weigand (1992) reported that the addition of microbial phytase to broiler diets increased Zn retention and decreased Zn excretion. More recently, Yi et al. (1996) reported that Zn retention from a low Zn diet (20 mg of Zn/kg) increased linearly in chicks fed increasing levels of phytase (150 to 600 U/kg of diet). These data taken together with results of the current study and that of Guaiume et al. (2002) suggest that a reduction in phytate content of diets, whether by using LP grains or supplemental phytase, should improve Zn utilization.

The decrease in Zn retention with increasing dietary Zn concentrations observed in the present study has been reported previously in chicks (Yi et al., 1996; Mohanna and Nys, 1999). Mohanna and Nys (1999) reported that only 8% of ingested Zn was retained when the diet contained 170 mg of Zn/kg, whereas Zn retention was 29, 25, and 19% in diets containing 20, 30, and 60 mg of Zn/kg, respectively. In the present study, Zn retention averaged 43% across all dietary treatments for both experiments, where the highest dietary Zn concentration was 48 mg/kg Zn.

A significant barley type × Zn level interaction also was observed for Zn retention in both experiments. Chicks fed both barley types showed a decrease in Zn retention when Zn supplementation was increased. However, the decrease in Zn retention was much greater in chicks fed LPB compared with those fed WTB. It appears that when Zn is limiting, more Zn is retained in the body for main-

Table 8. Effects of autoclaving of wild-type barley (WTB) and low phytate barley (LPB) and levels of added dietary Zn on retention of P, Ca, and Zn in broilers to 21 d of age (experiment 2)[1]

Barley type	Autoclaved	Zn (mg/kg)	P retention (%)	Ca retention (%)	Zn retention (%)
WTB	No	0	63.51	51.99	42.01
WTB	No	10	60.90	53.74	41.70
WTB	No	20	59.98	43.01	36.64
WTB	Yes	0	63.51	56.45	44.34
WTB	Yes	10	60.90	46.25	36.48
WTB	Yes	20	59.98	42.40	33.33
LPB	No	0	69.37	55.02	62.87
LPB	No	10	72.85	59.16	46.64
LPB	No	20	73.11	52.09	40.96
LPB	Yes	0	69.37	48.75	63.09
LPB	Yes	10	72.85	50.19	43.01
LPB	Yes	20	73.11	47.14	37.75
Pooled SEM			2.72	3.52	4.35
Source of variation				*P*-value	
Barley (B)			<0.0001	0.1375	0.0003
Autoclaved (A)			0.8388	0.0585	0.4018
Zn			0.9733	0.0162	<0.0001
B × A			0.8940	0.1833	0.9761
B × Zn			0.1417	0.1678	0.0306
A × Zn			0.5228	0.3223	0.6213
B × A × Zn			0.7304	0.6403	0.9545
Main effect mean					
B					
WTB			61.46[b]	48.97	39.09[b]
LPB			71.78[a]	52.06	49.05[a]
A					
No			66.78	52.50	45.14
Yes			66.46	48.53	43.01
Zinc	0		66.44	53.05[a]	53.09[a]
	10		66.87	52.34[a]	41.96[b]
	20		66.55	46.16[b]	37.17[b]

[a,b]Values within a column with no common superscript are significantly different ($P < 0.05$).

[1]Data are means of 4 replicate pens of 5 chicks per pen.

taining physiological functions, thus resulting in less Zn excretion in the waste. Baer and King (1984) and Wada et al. (1985) suggested that humans rapidly reduce Zn excretion in response to low Zn intake, and Ziegler et al. (1989) noticed that infants were able to increase Zn absorption efficiency and decrease excretion of endogenous Zn, thereby maintaining Zn balance, despite a drastic decrease in Zn intake. Also, increased Zn retention efficiency has been shown in rats (Huber and Gershoff, 1970) and in chicks (Emmert and Baker, 1995) with decreased Zn intakes. The results of the present study confirmed data from Guaiume et al. (2002), who also observed an interaction and main effects of Zn level and barley type in chicks fed LPB and WTB barley to 21 d.

Calcium retention in this study was not affected by substituting LPB for WTB in the diets. Similar results were also reported in turkeys (Li et al., 2001b), pigs (Veum et al., 2001), and fish (Sugiura et al., 1999) fed LPB and WTB. However, contrasting results were reported in fish fed LPB (Overturf et al., 2003) and in humans fed LP corn (Hambidge et al., 2005). The increase in Ca availability and absorption in the studies by Overturf et al. (2003) and Hambidge et al. (2005) are probably a consequence of the lower dietary Ca concentrations used in those studies. In this study, Ca retention declined with increasing Zn supplementation (0 mg/kg, 53.05%; 10 mg/kg, 52.34%; 20 mg/kg, 46.16%) indicating that Zn depressed Ca utilization, probably through the formation of an insoluble Ca-phytate-Zn complex, which decreases the absorption of both minerals (Kornegay, 2001).

No main or interactive effects of autoclaving were observed for any response variable. This may have occurred because of the low levels of endogenous phytase activity (183 and 190 PPU/kg for WTB and LPB, respectively) in the barley cultivars. Barley was the only ingredient that contained endogenous phytase activity, and when added at 60% in diets, it contributed with endogenous phytase of 110 PPU/kg for WTB and 114 PPU/kg for LPB diets. Thus, there was very little difference in phytase activity between the 2 barley diets. In addition, Eeckhout and De Paepe (1994) reported that plant phytase is only 58% as effective as microbial phytase, which would mean that the phytase activity (relative to microbial phytase) in these diets was only 64 and 66 PPU/kg, respectively. These levels of phytase may not have been high enough to cause improvements in mineral utilization. Therefore, it is not surprising that there were no main or interactive effects of autoclaving. In swine diets containing 482 PPU/kg of endogenous phytase activity from feedstuffs such as wheat, wheat middlings or barley, steam pelleting at approximately 80°C decreased the absorption of P and Ca by 10%. This was mainly due to a considerable reduc-

tion in the phytase activity (Jongbloed and Kemme, 1990). Edwards et al. (1999) observed no improvements in phytate P utilization by broilers when corn-soybean meal diets were pelletized or extruded. Using an in vitro procedure, Zyla et al. (1999) observed that autoclaving feed containing 55% wheat for 20 min remarkably decreased the amounts (76% reduction) of P liberated from the diet containing 282 to 377 PPU/kg of endogenous phytase activity.

Results of these studies indicated that chicks fed LPB were able to utilize more dietary P and Zn than chicks fed WTB. Therefore, diets containing LPB may not need to be supplemented with as much inorganic P and Zn. The combination of low levels of supplementary inorganic P and Zn and increased availability of both minerals in LPB may result in a significant reduction in P and Zn in poultry manure. Reduction of minerals in manure would contribute to a reduction in the potential for environmental pollution. Differences in Zn and P utilization by chicks fed WTB and LPB diets are due to differences in phytic acid content of the barleys and not due to endogenous phytase activity present in the grains.

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REFERENCES

Adams, C. L., M. Hambridge, V. Raboy, J. A. Dorsch, L. Sian, J. L. Westcott, and N. F. Krebs. 2002. Zinc absorption from a low-phytic acid maize. Am. J. Clin. Nutr. 76:556–559.

AOAC. 2000. Official Methods of Analysis. Assoc. Off. Anal. Chem., Washington, DC.

Atwal, A. S., N. A. M. Eskin, B. E. McDonald, and M. Vasey-Genser. 1980. The effects of phytate on nitrogen utilization and zinc metabolism in young rats. Nutr. Rep. Int. 21:257–267.

Baer, M. T., and J. C. King. 1984. Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. Am. J. Clin. Nutr. 39:556–570.

Biehl, R. R., D. H. Baker, and H. F. DeLuca. 1995. 1α-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese in chicks fed soy-based diets. J. Nutr. 125:2407–2416.

Cheryan, M. 1980. Phytic acid interactions in food systems. Crit. Rev. Food Sci. Nutr. 13:297–335.

Dorsch, J. A., A. Cook, K. A. Young, J. M. Anderson, A. T. Bauman, C. J. Volkmann, P. P. N. Murthy, and V. Raboy. 2003. Seed phosphorus and inositol phosphate phenotype of barley low phytic acid genotypes. Phytochem. 62:691–706.

Edwards, H. M., Jr., A. B. Carlos, A. B. Kasim, and R. T. Toledo. 1999. Effects of steam pelleting and extrusion on feed on phytate phosphorus utilization in broiler chickens. Poult. Sci. 78:96–101.

Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47:19–29.

Egli, I., L. Davidsson, C. Zeder, T. Walczyk, and R. Hurrell. 2004. Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults. J. Nutr. 134:1077–1080.

Emmert, J. L., and D. H. Baker. 1995. Zinc stores in chickens delay the onset of zinc deficiency symptoms. Poult. Sci. 74:1011–1021.

Garcia, A. R., and N. M. Dale. 2006. Foot ash as means of quantifying bone mineralization in chicks. J. Appl. Poult. Res. 15:103–109.

Guaiume, E. A., J. N. Broomhead, D. R. Ledoux, T. L. Veum, V. Raboy, and K. Zyla. 2002. Zinc utilization is improved in broiler chicks fed a diet containing low phytic acid barley (*Hordeum vulgare* L.). Poult. Sci. 81(Suppl. 1):142. (Abstr.)

Hambidge, K. M., N. F. Krebs, J. L. Westcott, L. Sian, L. V. Miller, K. L. Peterson, and V. Raboy. 2005. Absorption of calcium from tortilla meals prepared from low-phytate maize. Am. J. Clin. Nutr. 82:84–87.

Huber, A. M., and S. N. Gershoff. 1970. Effects of dietary zinc and calcium on the retention and distribution of zinc in rats fed semipurified diets. J. Nutr. 100:949–954.

Jang, D. A., J. G. Fadel, K. C. Klasing, A. J. Mireles, Jr., R. A. Ersnt, K. A. Young, A. Cook, and V. Raboy. 2003. Evaluation of low-phytate corn and barley on broiler chick performance. Poult. Sci. 82:1914–1924.

Jongbloed, A. W., and P. A. Kemme. 1990. Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. Anim. Feed Sci. Technol. 28:233–242.

Kies, A. K., K. H. F. Van Hemert, and W. C. Sauer. 2001. Effect of phytase on protein and amino acid digestibility and energy utilization. World's Poult. Sci. J. 57:109–126.

Kornegay, E. T. 2001. Digestion of phosphorus and other nutrients: The role of phytases and factors influencing their activity. Pages 237–272 in Enzymes in Farm Animal Nutrition. CABI Publishing, New York, NY.

Lease, J. G. 1966. The effect of autoclaving sesame meal on its phytic acid content and on the availability of its zinc to the chick. Poult. Sci. 45:237–241.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444–1450.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, K. Zyla, and A. Wikiera. 2001a. Bioavailability of phosphorus in low phytic acid barley. J. Appl. Poult. Res. 10:86–91.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and K. Zyla. 2001b. Low phytic acid barley improves performance, bone mineralization, and phosphorus retention in turkey poults. J. Appl. Poult. Res. 10:178–185.

Linares, L. B., J. N. Broomhead, E. A. Guaiume, D. R. Ledoux, T. L. Veum, and V. Raboy. 2003. Low phytate barley and phytase reduces phosphorus excretion in broilers fed diets for 42 days. Poult. Sci. 82(Suppl. 1):94. (Abstr.)

Lo, G. S., S. L. Settle, F. H. Steinke, and D. T. Hopkins. 1981. Effect of phytate:zinc molar ratio and isolated soybean protein on zinc bioavailability. J. Nutr. 11:2223–2235.

Lonnerdal, B., A. S. Sandberg, B. Sandstrom, and C. Kunz. 1989. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. J. Nutr. 119:211–214.

Mohanna, C., and Y. Nys. 1999. Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. Br. Poult. Sci. 40:108–114.

Morris, E. R. 1986. Phytate and dietary mineral bioavailability. Pages 57–76 in Phytic Acid Chemistry and Application. Pilatus, Minneapolis, MN.

Morris, E. R., and R. Ellis. 1980. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J. Nutr. 110:1037–1045.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Sci., Washington, DC.

Oberleas, D., and B. F. Harland. 1996. Impact of phytic acid on nutrient availability. Pages 77–84 in Phytase in Animal Nutrition and Waste Management. BASF Corp., Mount Olive, NJ.

Oberleas, D., M. E. Muher, and B. L. O'Dell. 1966. Dietary metal-complexing agents and zinc availability in the rat. J. Nutr. 90:56.

Ockenden, I., J. A. Dorsch, M. M. Reid, L. Lin, L. K. Grant, V. Raboy, and J. N. A. Lott. 2004. Characterization of the storage of phosphorus, inositol phosphate and cations in grain tissues of four barley (*Hordeum vulgare* L.) low phytic acid genotypes. Plant Sci. 167:114.

O'Dell, B. L., and J. E. Savage. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med. 103:304–306.

Overturf, K., V. Raboy, Z. J. Cheng, and R. W. Hardy. 2003. Mineral availability from barley low phytic acid grains in rainbow trout (*Oncorhynchus mykiss*) diets. Aquaculture Nutrition 9:239–246.

Potter, L. M., M. Potchanakorn, V. Ravindran, and E. T. Kornegay. 1995. Bioavailability of phosphorus in various phosphate sources using body weight and toe ash as response criteria. Poult. Sci. 74:813–820.

Raboy, V. 2001. Seeds for a better future: 'Low phytate' grains help to overcome malnutrition and reduce pollution. Trends Plant Sci. 6:458–462.

Ravindran, V., W. L. Bryden, and E. T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. Rev. 6:125–143.

SAS. 1996. Statistics. In SAS User's Guide, 1996 ed. SAS Institute, Cary, NC.

Sugiura, S. H., V. Raboy, K. A. Young, F. M. Dong, and R. W. Hardy. 1999. Availability of phosphorus and trace minerals in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 170:285–296.

Thiel, U., and E. Weigand. 1992. Influence of dietary zinc and microbial phytase supplementation on zinc retention and zinc excretion in broiler chicks. Page 460 in Proc. World's Poult. Congr. Vol. 3. World's Poult. Sci. Assoc., Amsterdam, the Netherlands.

Veum, T. L., D. R. Ledoux, V. Raboy, and D. S. Ertl. 2001. Low-phytic acid corn improves nutrient utilization for growing pigs. J. Anim. Sci. 79:2873–2880.

Wada, L., J. R. Turnland, and J. C. King. 1985. Zinc utilization in young men fed adequate and low zinc intakes. J. Nutr. 115:1345–1354.

Waldroup, P. W., J. H. Kersey, E. A. Saleh, C. A. Fritts, F. Yan, H. L. Stilborn, R. C. Crum, Jr., and V. Raboy. 2000. Nonphytate phosphorus requirements and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci. 79:1451–1459.

Yan, F., J. H. Kersey, C. A. Fritts, P. W. Waldrup, H. L. Stilborn, R. C. Crum, Jr., D. W. Rice, and V. Raboy. 2000. Evaluation of normal yellow dent corn and high available phosphorus corn in combination with reduced dietary phosphorus and phytase supplementation for broilers grown to market weights in litter pens. Poult. Sci. 79:1282–1289.

Yi, Z., E. T. Kornegay, and D. M. Denbow. 1996. Supplemental microbial phytase improves zinc utilization in broilers. Poult. Sci. 75:540–546.

Ziegler, E. E., R. E. Serfass, S. E. Nelson, R. Figueroa-Colon, B. B. Edwards, R. S. Houk, and J. J. Thompson. 1989. Effect of low zinc intake on absorption and excretion of zinc by infants studied with 70Zn as extrinsic tag. J. Nutr. 119:1647–1653.

Zyla, K., D. Gogol, J. Koreleski, S. Swiatkiewicz, and D. R. Ledoux. 1999. Simultaneous application of phytase and xylanase to broiler feeds based on wheat: In vitro measurements of phosphorus and pentose release from wheats and wheat-based feeds. J. Sci. Food Agric. 79:1832–1840.

Zyla, K., M. Czubak, J. Roznowski, and T. Fortuna. 2002. Changes in the activity of endogenous phytase in germinating sesame seeds. Pages 137–138 in Proc. XXXIII Sci. Mtg. Food Chem. Technol. Committee of Polish Acad. Sci., Lublin, Poland.